Fragile X Syndrome: Keys to the Molecular Genetics of Synaptic Plasticity

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In the previous column, we briefly discussed fragile X syndrome. Here, we expand on this developmental disorder because it introduces key aspects of synaptic plasticity that are the focus of several future columns. Fragile X syndrome is the most common form of inherited mental retardation. Its clinical symptoms include moderate to severe mental retardation, macroorchidism, and behavioral symptoms that include hyperactivity, gaze aversion, and stereotypies. A fragile site on the X chromosome was discovered in 1969, when magnified views of X chromosomes from affected individuals suggested that a section at the end of the chromosome had separated from the rest of the chromosome.

The explanation for the fragile site came with the cloning and characterization of the fragile X mental retardation 1 gene (*FMR1)* in 1991. Gene sequencing indicates that a portion of the gene is dramatically expanded, with sometimes 1,000 to 2,000 new nucleotides found in affected individuals. The new sequence is of a particular sort: the repeat of the three nucleotides cytosine, guanine, and guanine (called a CGG repeat). The expansion is located immediately adjacent to the regulatory portion of the gene, termed the promoter, and interferes with normal transcription of the gene. The net effect is that little if any message is produced, and little if any protein is translated. The gene is effectively silenced.

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All humans have a small number of CGG repeats at this site, with between 5 and 50 repeats normally present throughout the population. This short repeated sequence does not interfere with transcription. However, the repeat expands to between 50 and 200 repeats in some individuals. Why this happens is not yet understood, but when it occurs, the repeated element expands in the next generation. Individuals with the initial expansion of 200 repeats are carriers, who were initially thought to be affected only mildly, if at all. However, more recent studies show that carriers often develop fragile X tremor ataxia syndrome characterized by gait ataxia, tremor on the onset of movements, as well as milder cognitive and neurological symptoms.

Fragile X syndrome is the second disorder in which an expanded series of nucleotides is found in affected patients. Another triplet repeat occurs in Huntington's chorea. That disorder is characterized by the expansion of a different triplet repeat (cytosine, adenosine, and guanine [CAG]). Moreover, the expansion occurs within the coding sequence of the Huntington gene and leads to the inappropriate addition of amino acids to the expressed protein. This novel stretch of 10 to 30 glutamines is sufficient to disrupt the normal function of the huntingtin protein, as it is now called.

Over the past 15 years, triplet repeat expansions have been found in more than a dozen disorders in addition to fragile X syndrome; these disorders include Huntington's chorea, Friedreich's ataxia, and myotonic dystrophy (Fig. 1). Several generalizations can be made about triplet repeat expansions in these disorders. First, triplet repeats can occur in any of the four nucleotides, although several are more commonly found than others (e.g., CGG or CAG). Second, the sites of the expansions are found in different locations within a particular gene, depending on the disorder. If the expansion occurs within the coding sequence, as it does in Huntington's

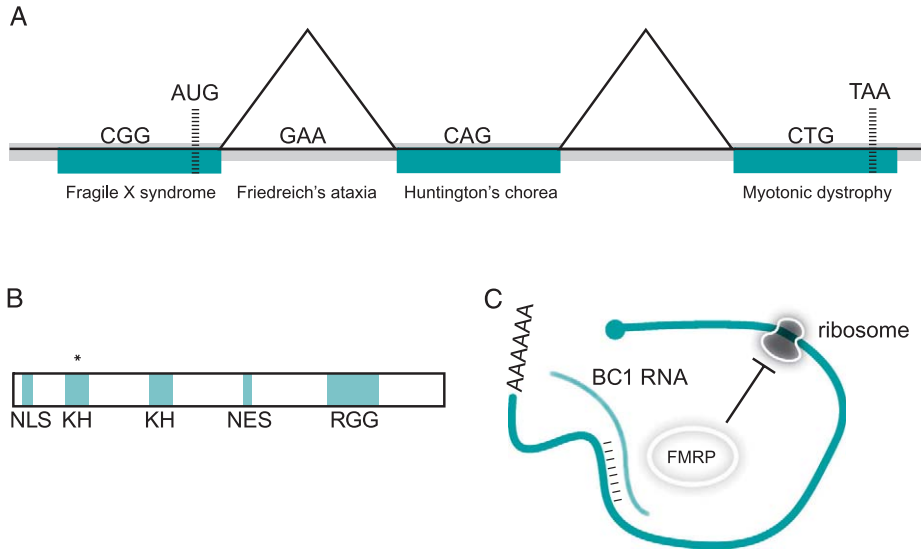

Fig. 1 A, Triplet repeats can be found in several portions of a gene. Shown is a hypothetical gene with three exons separated by two introns. The thin lines indicate exons spliced together to form mature mRNA. A start codon (AUG) and stop codon (TAA) represent the first and last amino acids, respectively, that will be translated into the encoded protein. The triplet repeat of fragile X syndrome is found within the 5 untranslated region of the message. In Friedreich's ataxia, the triplet repeat lies within an intron and is thought to disrupt proper splicing of the message. In Huntington's disease, the triplet repeat is found within the coding sequence itself and results in a large tract of glutamines added to the protein that disrupts its normal function. In myotonic dystrophy, the triplet repeat occurs within the 3 untranslated region of the message, and whether it disrupts the upstream processing of the gene or of a nearby downstream gene remains unclear. B, Several amino acid domains have been characterized within the fragile X mental retardation protein; three RNA-binding domains, two K homology (KH) domains, and an arginine/glycine-rich domain (RGG box) have been shown to interact with mRNAs. In addition, a nuclear localization signal (NLS) and a nuclear export signal (NES) help to move the fragile X mental retardation protein (FMRP) complex from the nucleus out to the cytoplasm and back. Most cases of fragile X syndrome are caused by a triplet repeat expansion. Mutations in critical domains, such as the RNA-binding domain, have been found in affected individuals (asterisk). C, Fragile X mental retardation protein (FMRP) forms a complex that includes a small, noncoding RNA molecule (BC1) that associates through complementary sequences with selected mRNA molecules that are transported along dendrites to lie near synaptic spines. The complex is thought to prevent translation of the message until an incoming signal arrives at the spine. Exactly how FMRP is then released and how local translation of the message is initiated is an area of active research.

chorea, then the inappropriate addition of a novel amino acid disrupts protein function. If the expansion occurs adjacent to the promoter region, as is seen in fragile X syndrome, then gene transcription is disrupted. If the expansion occurs in intervening sequences (introns) that lie between the coding sequences (exons) of a gene, then the disruption involves message processing (splicing). Finally, in myotonic dystrophy, the expansion occurs toward the end of the gene, and how expansion at this site disrupts gene function remains unknown.

The expansion of a triplet repeat explained a genetic phenomenon called anticipation that had been known for many years. Anticipation refers to a genetic disorder that becomes more severe over several generations. It had been thought that ascertainment bias was the reason for this observation. Anticipation is now explained by the progressive increase in size of the triplet repeat as it is passed from parent to child. In some instances, anticipation can be dramatic. For example, in myotonic dystrophy, a great-grandfather may simply have cataracts late in life, but members of the next several generations display progressively more severe and earlier onset of motor symptoms.

But let us return to fragile X syndrome. Women carriers with 200 or so repeats bear children with a large expansion of the CGG repeat, and these children develop fragile X syndrome. There are two consequences of the CGG expansion. The first is that the expansion assumes a secondary structure consisting of hundreds of hairpin turns. In addition, as mentioned in the last column, CGG repeats are often the target of a chemical modification called methylation, and methyl groups are added to the greatly increased number of cytosines. Methylation is an epigenetic modification that often occurs within regulatory regions of a gene to control the expression of that gene. This happens when the methylated cytosine binds to and recruits a complex of proteins whose function is to compress the underlying chromatin. The highly compacted DNA is then neither accessible to transcription factors nor to RNA polymerase, which normally binds to

the promoter and initiates transcription. Thus, no mRNA is produced and no functional protein is translated. It is interesting to note that the expansion also explains the appearance of the fragile site in fragile X syndrome; the tightly wound DNA prevents staining by dyes that are normally used during karyotyping, leading to the characteristic appearance of a break in the chromosome.

When the *FMR1* gene was cloned, researchers were able to focus on determining the normal function of the fragile X mental retardation protein (FMRP). A corollary to this important question is how the absence of this protein leads to cognitive deficits. We first describe what has been discovered during the past decade regarding the function of FMRP and how it plays a central role in synaptic plasticity. The consequences of silencing this gene and the potential to reverse this condition are discussed in the next column.

Once the fragile X gene had been sequenced, investigators could study the amino acid sequence that it encoded. Several amino acid motifs in the fragile X protein were immediately recognized as domains that are present in families of proteins with known functions. Two domains are termed K homology domains (KH), and the third is enriched in arginines and glycines residues (RGG domain). Proteins that contain these domains are RNA-binding proteins. Their presence in FMRP suggested a possible function for the protein, implicating FMRP as a new member of this large family of proteins. This was a testable hypothesis, and investigators soon showed that FMRP could indeed bind to RNA molecules. In addition, researchers found a patient with severe fragile X syndrome but without the classic repeat expansion. Instead, the patient had a point mutation in one of his two KH domains. His FMRP protein was unable to bind to RNA molecules, suggesting a critical function of the RNA-binding motif and implicating the loss of this function with the clinical disorder.

Two additional amino acid sequences are present in FMRP. The first is a nuclear export signal, and proteins that contain this sequence are exported out of the nucleus and into the cytoplasm. The second is a nuclear localization signal, and proteins that contain this sequence are transported into the nucleus. Taken together, these initial studies suggest that FMRP shuttles in and out of the nucleus transporting RNA molecules.

If FMRP is an RNA-binding protein, then identifying the associated RNA molecules would be an important step forward. Several groups turned their attention to this goal. They made use of antibodies generated against FMRP to pull down, or immunoprecipitate, the FMRP complex consisting of FMRP itself and any associated proteins or RNA molecules. Once immunoprecipitated, the associated mRNAs were converted to their more stable complementary sequence (cDNAs), and the cDNAs were fluorescently tagged and used as probes to screen microarrays.

Microarrays are small chips to which many thousands of DNA molecules have been robotically attached. The exact position of each DNA molecule is known, as is the identity of its sequence. The fluorescently tagged probes isolated from the FMRP immunoprecipitation experiments were added onto the microarrays. Because complementary nucleic acid sequences bind tightly to each other, the FMRP-associated messages were used to identify any sequences attached to the chip. The microarrays could then be scanned and the identity of the FMRP-associated mRNAs determined. In this manner, a handful of mRNAs were soon discovered.

FMRP uses several mechanisms to bind to its target mRNAs. FMRP was first shown to interact with a G quartet, a series of guanines present in the target message that binds to one of the RNA-binding domains of FMRP. FMRP also associates with a small noncoding RNA molecule (BC1). BC1 is approximately 150 nucleotides long and contains sequences that are complementary to specific messages. In this way, a complex is formed that consists of FMRP associated with BC1, whereas BC1 binds to the mRNAs. It is this complex of proteins and mRNAs that is transported down dendrites to the spines, where they await an incoming signal that will initiate local protein synthesis.

To fully appreciate the importance of this last statement, remember that the existing dogma at the time was that messages are synthesized in the nucleus and transported to the perinuclear cytoplasm. It is only after they are in the cytoplasm that ribosomes bind to the messages and translate them into proteins. The new model suggested that some messages are actually transported down dendrites to distant spines where they are subsequently translated. This novel process requires the presence at spines of all of the machinery necessary for the translation of messages into proteins. In fact, careful electron microscopy studies performed in the 1980s showed that ribosomes and proteins known to be necessary for translation are present at distant

regions of neurons called spines. Because these data contradicted the accepted dogma, they were not fully appreciated at the time.

This new model also helped solve another conundrum. Synaptic plasticity refers to the strengthening of synaptic contacts between neurons. It allows an incoming signal to produce a stronger response in the postsynaptic neuron, thus increasing synaptic efficacy. One can imagine several mechanisms that could accomplish this. For example, the presynaptic terminal may release more neurotransmitter for any given action potential arriving at the terminal, or the postsynaptic terminal could place greater numbers of receptors at the active synapse. Finally, receptors that are present at the active site may be made more responsive to a given amount of released neurotransmitter. In fact, there is ample evidence of each of these mechanisms. The net effect is that repeated use of a synapse strengthens the synaptic connection. Repeated signaling causes a more pronounced response to a given action potential than before the repeated signaling. This increase in synaptic plasticity is reflected in structural changes in the spine itself. For example, where there was one spine, repeated synaptic activity may lead to the growth of a second spine at that location.

Several points are worth emphasizing here. The development of synaptic plasticity requires new proteins, both for the structural changes that occur in the spines and for the increased enzymatic activity within the activated spine. Moreover, this system requires specificity. That is, a defined number of spines should be strengthened and not the entire set of spines on a single dendrite. Because there are sometimes >10,000 spines on a given neuron, proteins synthesized in the cytoplasm around the nucleus (according to the old model) would have to somehow be delivered to only those 100 spines where synaptic plasticity was developing and not captured by other spines along the way. Thus, having the messages themselves present at the spines presents a beautiful solution to a complex problem. Messages could be transported throughout the dendrites and initially inhibited from translation. An incoming signal (synaptic activity) would initiate the local translation of those messages at only those spines at which they were needed for the morphological changes that accompany synaptic plasticity.

Several of the mRNAs that bind to FMRP encode proteins enriched within spines and known to be required for the development of synaptic plasticity. Examples include structural proteins (MAP2, PSD-95), receptors (glutamate receptor subunits), and proteins required for the translation of mRNA into proteins (elongation factor 1α).

To summarize, the present model is that FMRP regulates the transport and translation of messages whose protein products are required at the spine for the development of synaptic plasticity. Local protein synthesis occurs close to the actual site of synaptic stimulation. Thus, activation at the synapse leads to rapid triggering of local protein synthesis and the production of structural and signaling proteins. Not surprisingly, the development of synaptic plasticity is impaired in the absence of FMRP. The next column summarizes recent findings that demonstrate specific impairments in synaptic plasticity in a mouse model of fragile X syndrome and the potential for therapeutic interventions.

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